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Our research program aims to understand the mechanisms by which major neurotransmitter pathways regulate the biological clock in the suprachiasmatic nucleus (SCN) of the mammalian brain. Our model species is the rat. The specific progress made by each of the three students supported by the AASERT award in FY1 is summarized below. Each of these students has maintained satisfactory grades and progress toward their degree requirements during the funding period.

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## AASERT Technical Report

### (FY92 AASERT) AUGMENTATION OF RESEARCH TRAINING IN CHRONOBIOLOGY: REGULATION OF THE MAMMALIAN CIRCADIAN CLOCK BY NEUROTRANSMITTERS

Grant No. F49620-93-1-0413

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Our research program aims to understand the mechanisms by which major neurotransmitter pathways regulate the biological clock in the suprachiasmatic nucleus (SCN) of the mammalian brain. Our model species is the rat. The specific progress made by each of the three students supported by this AASERT award in FY91 is summarized below. Each of these students has maintained satisfactory grades and progress toward their degree requirements during the funding period.

**STEVEN M. DEMARCO:** GAD is the key biosynthetic enzyme for GABA, a major inhibitory neurotransmitter in the SCN. Steve examined the hypothesis that the concentration and specific activity of glutamic acid decarboxylase (GAD) in SCN are under circadian clock regulation. By probing Western blots of SCN proteins separated by polyacrylamide gel electrophoresis with antibodies specific to the two major isoforms, Steve demonstrated that both GAD65 and GAD67 are expressed in the SCN. They are present throughout the diurnal cycle, at circadian times (CTs) 4, 10, 16 and 22, in nearly equal concentrations. These isoforms paralleled each other in spontaneous circadian changes in abundance: They were high daily at CTs 10 and 22, and low at CT 16 (Tukey 1-way ANOVA,  $p \leq .005$ ). Specific activity was evaluated at eight points in the 24-h cycle. Pyridoxyl phosphate (PLP)-stimulated activity exhibited significant highs at CT 10 and 19 ( $p \leq .04$ ), where as this cofactor-stimulated activity expressed a significant low at CT 4. These results suggest that there is circadian modulation of GAD activity, which is partially under the control of the cofactor. Interestingly, levels and activity both peaked in late day and late night. These two times may represent different inhibitory states for the SCN, one acting within neurons and circuits within this structure (CT 10), and another acting external to inhibit efferent targets as the circadian system moves into the inactive period for this nocturnal rodent (CT 19/22). Steven DeMarco was awarded his M.S. in Biology from the University of Illinois in May, 1994 and has joined a neuroimmunology doctoral program at the Mayo School for Graduate Research, Rochester, MN.

**MARIJA MEDANIC:** Marija has continued her investigation of the regulatory role of serotonin (5HT) and neuropeptide Y (NPY) upon the phasing of the SCN clock. Information from other brain regions to SCN carried by these neurotransmitter systems is thought to convey information about photic changes and behavioral arousal states during the daytime portion of the cycle. Afferents from the raphe (5HT) and intergeniculate leaflet (NPY) terminate in ventrolateral SCN, often upon the same neurons. Both 5HT and NPY induce phase advance of SCN rhythms in daytime when applied alone. To investigate the hypothesis that integration of nonphotic modulatory signals occurs directly at the level of the SCN, Marija tested that ability of 5HT and NPY to alter the phase of the SCN when applied simultaneously to the SCN *in vitro*. Potential interactions were evaluated at CT 7 and CT 23, both points of sensitivity of the SCN to NPY.

SCN brain slices were treated with microdrops ( $10^{-11}$  ml) containing a fresh mixture of 5HT and NPY. The concentration of 5HT was kept constant at  $10^{-6}$  M, while varying the concentration of NPY. The effects of these treatments on the phase of the rhythm of electrical activity of the population of SCN neurons were assessed on the second day *in vitro*. While equimolar concentrations of NPY and 5HT caused phase advances of  $3.5 \pm 0.2$  h, the same phase shift as NPY alone, decreasing the NPY concentration resulted in the larger phase shifts that are characteristic of 5HT alone at CT 7. At CT 23, the shift was characteristic of NPY alone, with no effect of 5HT. This demonstrates that putative neurotransmitters for nonphotic zeitgebers can interact directly at the level of the SCN.

**THOMAS K. TCHENG:** Tom is continuing his efforts to develop a multiunit electrode that can simultaneously monitor the activity of a small population of SCN neurons. He has succeeded in modifying our usual brain slice system into one that automatically records the circadian oscillation in neuronal firing rate for at least 3 and more often 4-5 days *in vitro*. He has developed the computer software to acquire, store and analyze this data on line. He has used this system to compare the period of the free-running SCN circadian rhythm of neuronal activity with free-running behavioral rhythms in locomotory activity, drinking and temperature rhythms, which were measured in the behavioral monitoring system in Dr. Evelyn Satinoff's laboratory. He has found that the period of the SCN rhythm in the brain slice, which contains less than the entire SCN, matches very closely the organismic rhythms measured in freely behaving animals from our inbred rat colony. He is using this recording system to assess the hypothesis that glutamate, the putative neurotransmitter carrying information about light signals from the optic nerves to SCN, induces both acute and long-term effects upon groups of SCN neurons.